

Adapter-directed display systems

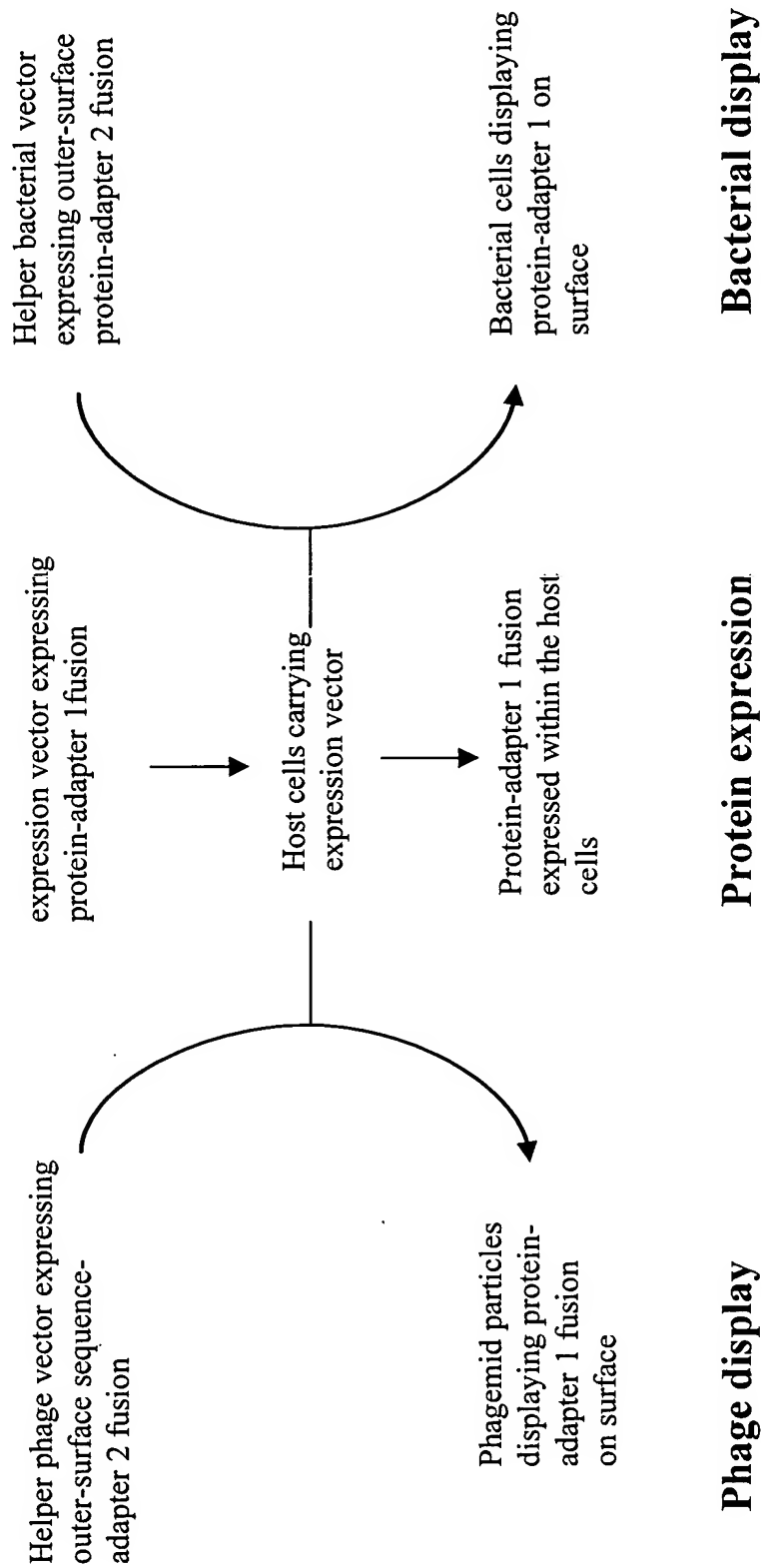


Fig. 1

KO7kpn phage Screening by ELISA

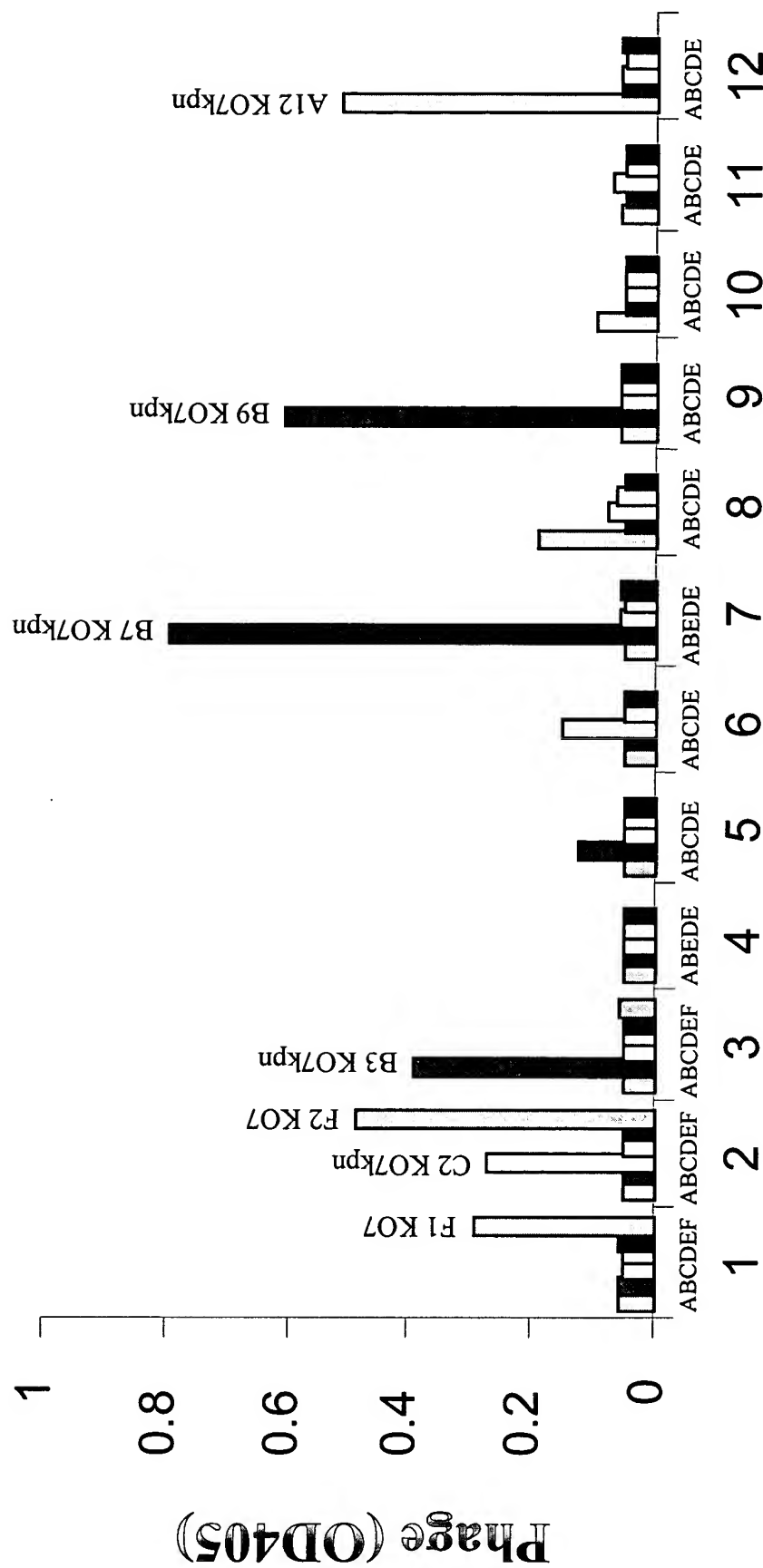


Fig. 2

KO7kpn helper phage Vector

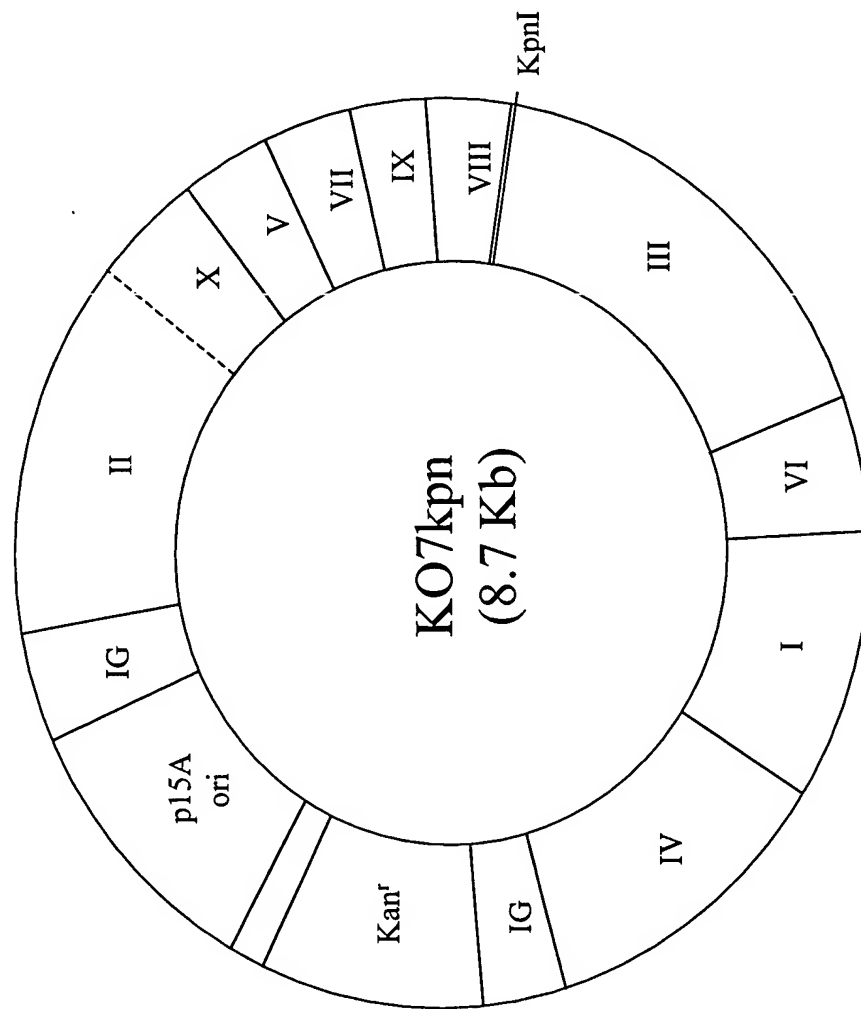


Fig. 3A

TCCTCTT" 156EECCTT

Gene III leader sequence in KO7 helper phage

GTG AAA AAA TTA TTC GCA ATT CCT TTA GTT GTT CCT TTC TAT TCT CAC TCC GCT
V K K L L F A I P L V V P F Y S H S A

Gene III leader sequence in KO7kpn helper phage

GTG AAA AAA TTA TTA TTC GCA ATT CCT TTA GTG GTA CCT TTC TAT TCT CAC TCC GCT
V K K L L F A I P L V V P F Y S H S A

KpnI

Fig. 3B

Map of phagemid vector pABMC6

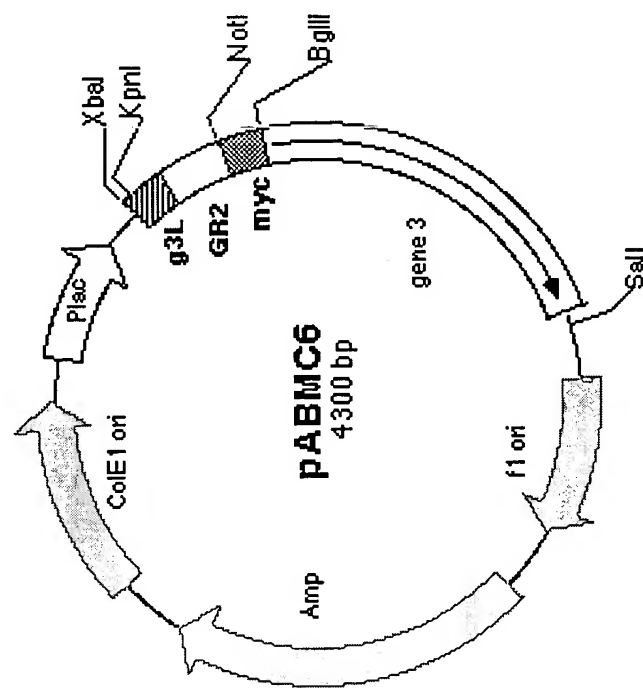


Fig. 4

Helper phage with engineered gene III fused to adaptor 2

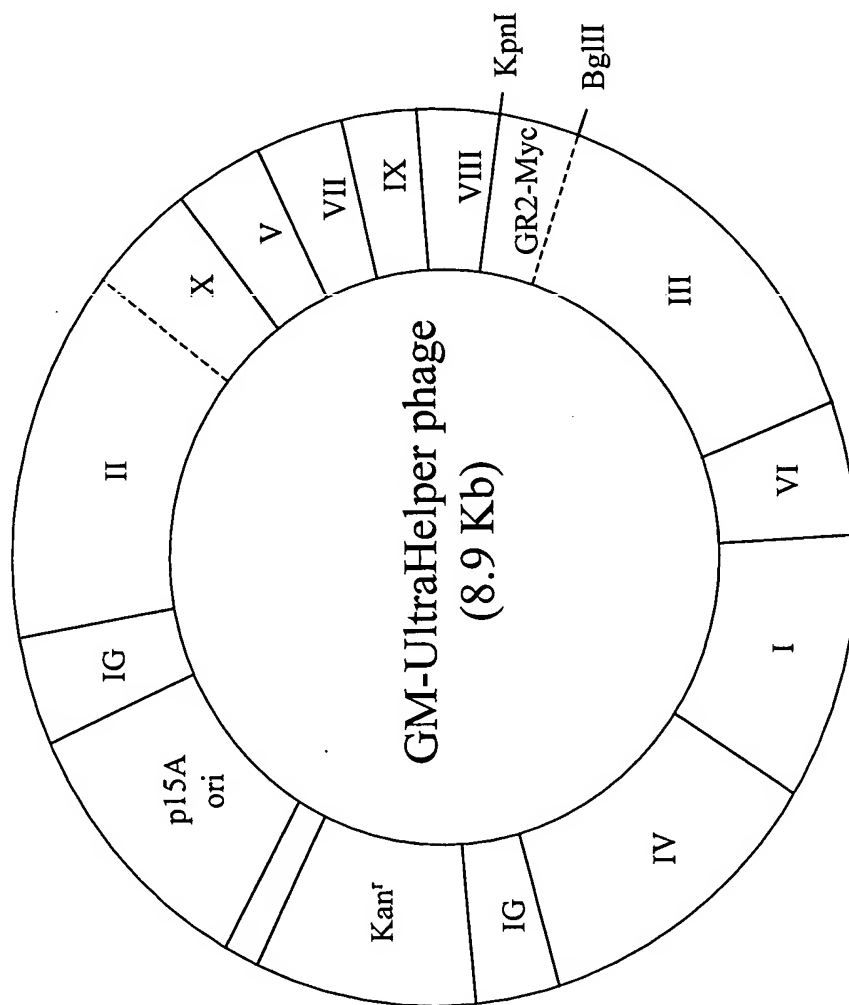


Fig. 5A

GR2-Myc domain coding sequence in GM-UltraHelper phage genome

KpnI	Gene III leader	GR2
---	TTAGTGGTACCTTTCTATTCTCACTCCGCT	ACATCCCGCCTGGAGGGCCCTACAGTCAGAAAAACCATCGCCTGCGA
-	L V V P F Y S H S A T S R L E G L Q S E N H R L R	
		NotI
	ATGAAGATCACAGAGCTGGATAAAGACTTGGAAAGAGGTCACCATGCAGCTGCAGGACGTCGGAGGTTGC	GCGGCCGCA
	M K I T E L D K D L E E V T M Q L Q D V G G C A A A	
	Myc-tag	Gene III
	BglII	
	GAACAAAACTCATCTCAGAAGAGGATCTG	AGATCTGGAGGCGGT ACTGTTGAAAGTTGTTAGCAAAA---
	E Q K L I S E E D L R S G G G T V E S C L A K -	

Fig. 5B

TO207T" 66EE00T

Trypsin cleavage sites at GR2-Myc domain on GM-UltraHelper phage

GR2 domain

T S R L E G L Q S E N H R L R M K I T E L D K D L E E V

Myc-tag

T M Q L Q D V G G C A A E Q K L I S E E D L R S G G G

Fig. 5C

GR2-Myc-pIII fusions assembly into GM phage particles

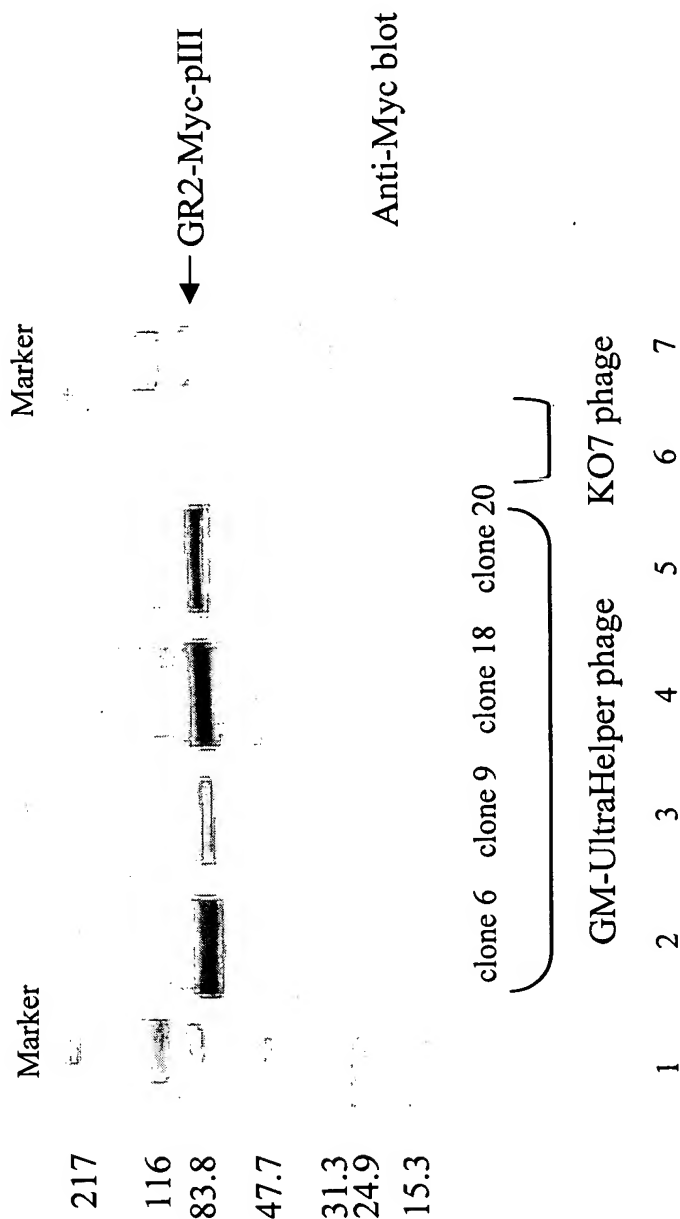


Fig. 6

Detection of GR2-Myc domain on GM-UltraHelper phage

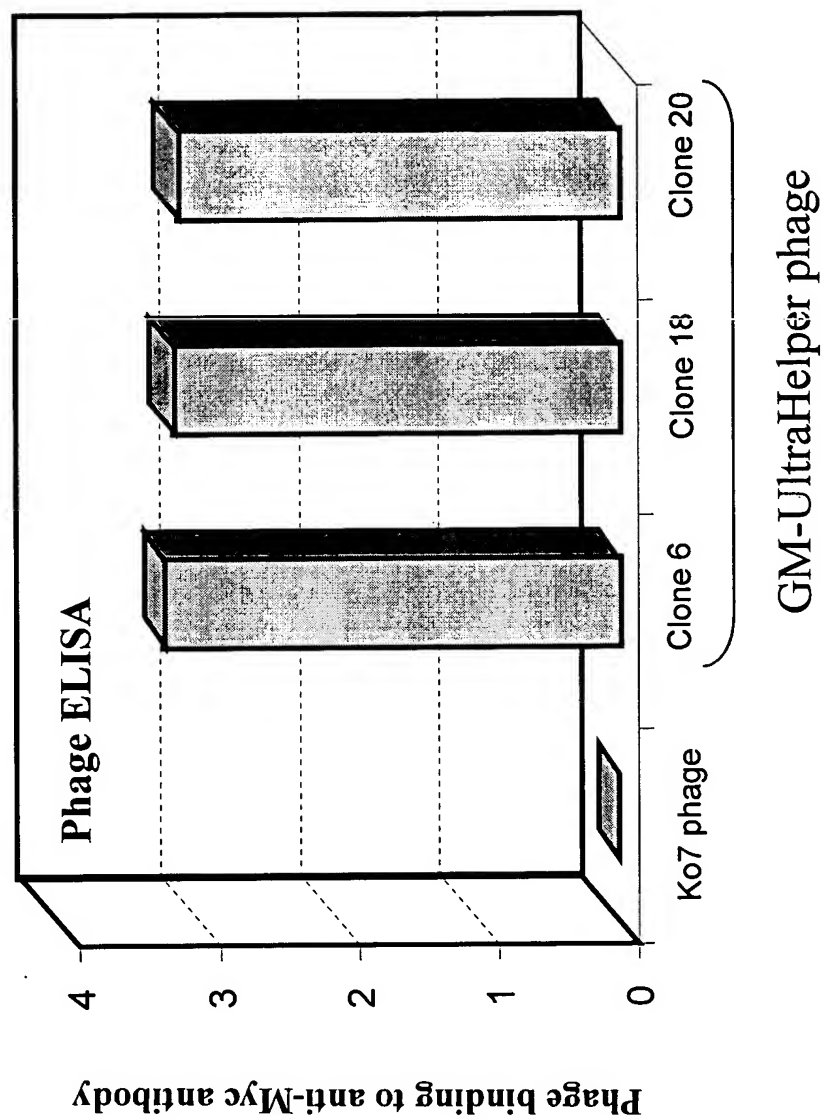


Fig. 7

Cleavage of GR2-Myc domains on GM phages by trypsin

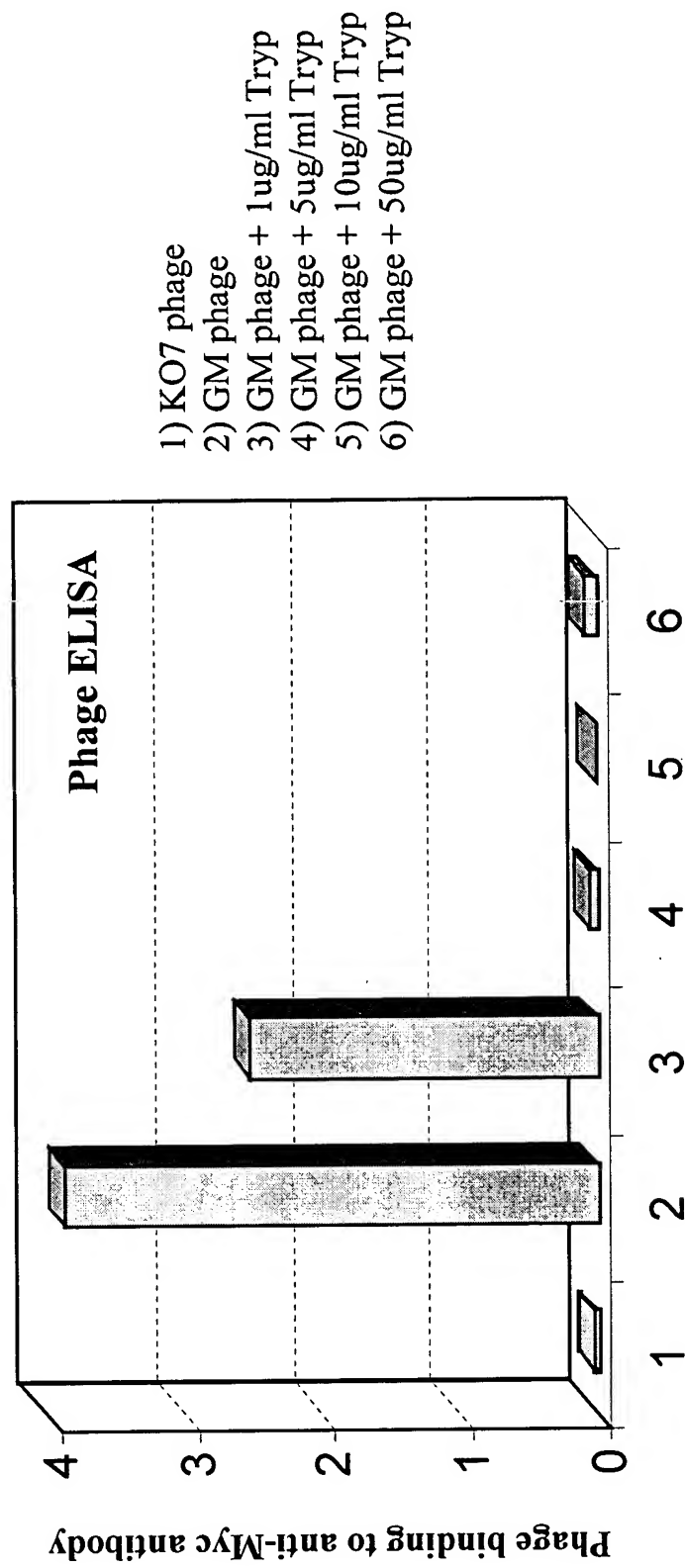


Fig. 8

Phagemid vector for protein-GR1 expression

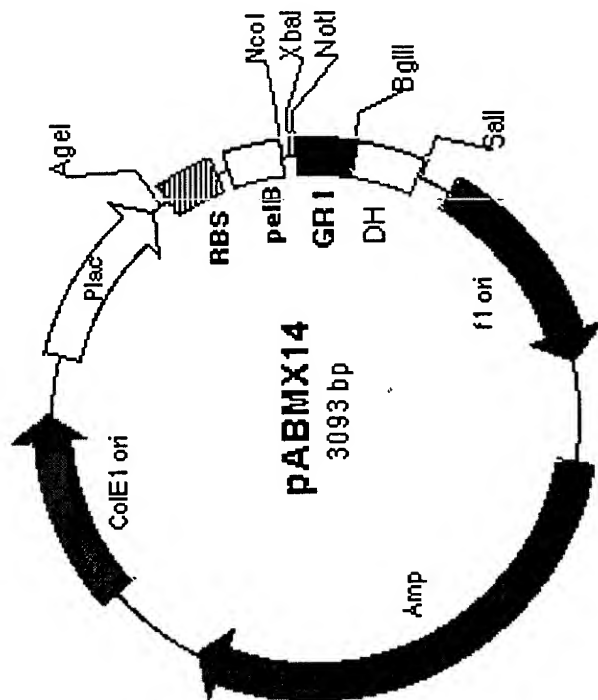


Fig. 9A

Complete vector sequence of pABMX14

[illegible]

Fig. 9B

Functional display of scFv by GM-UltraHelper phage

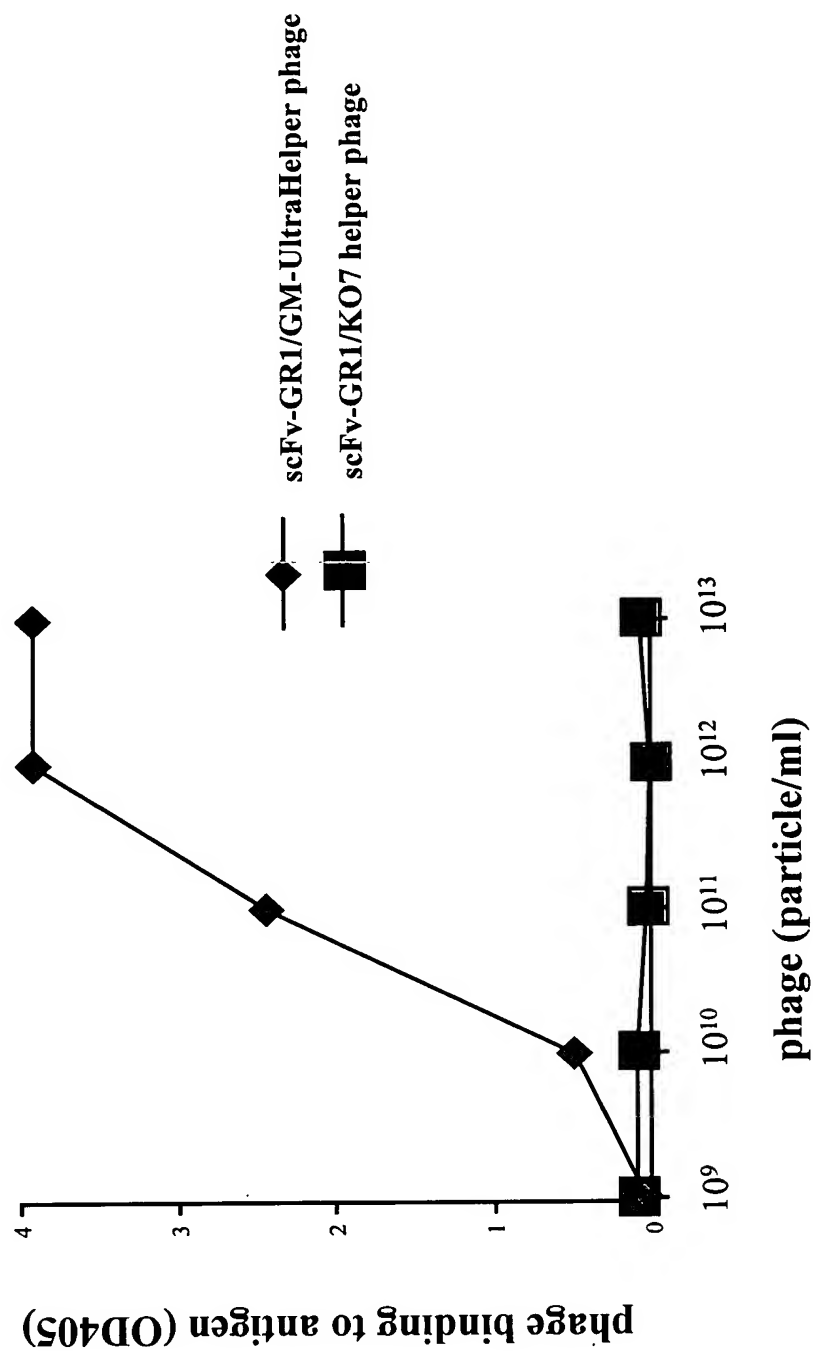


Fig. 10

Mutivalent display of scFv by GM-UltraHelper phage

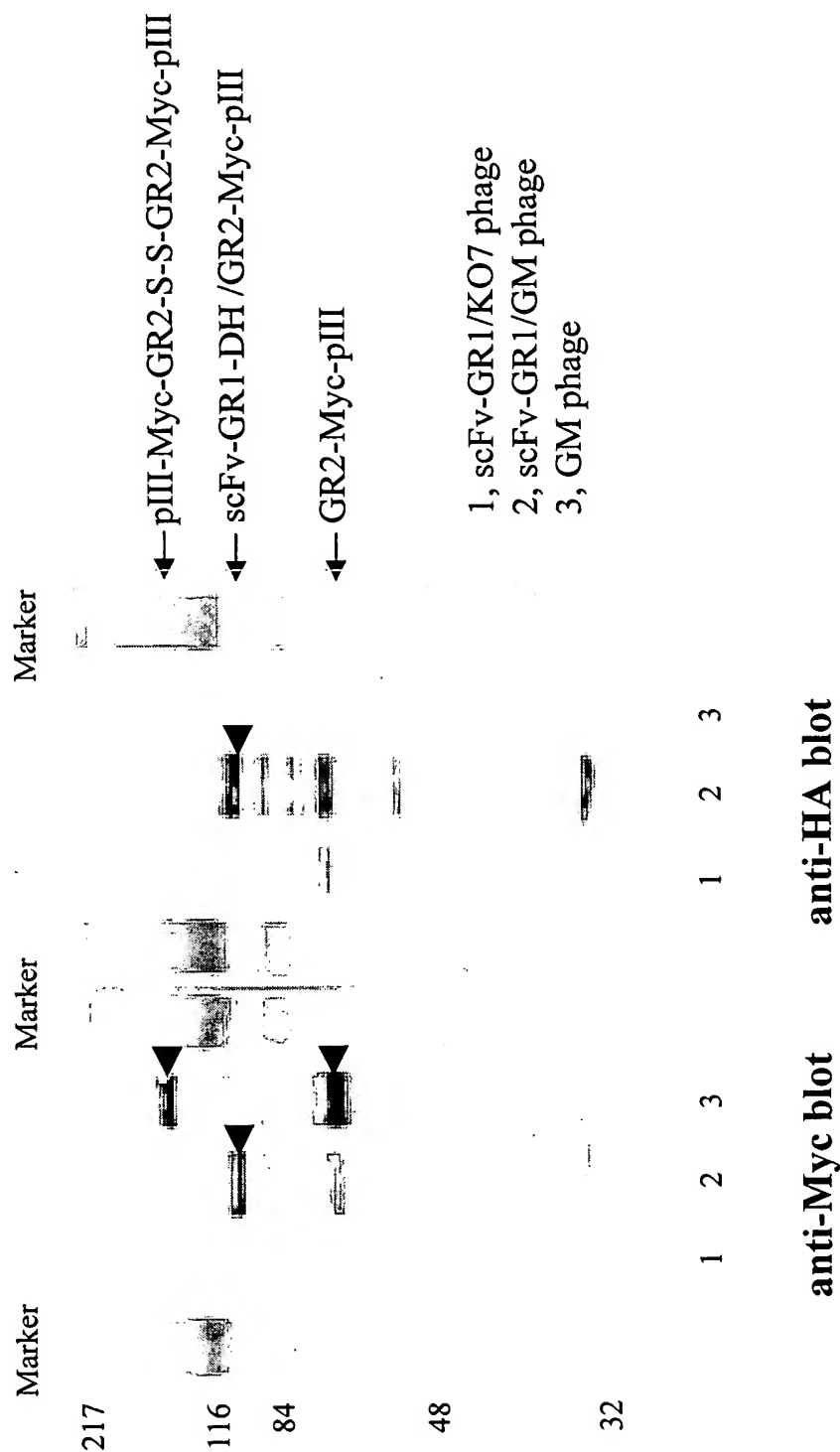


Fig. 11

Map of phagemid vector pABMC13

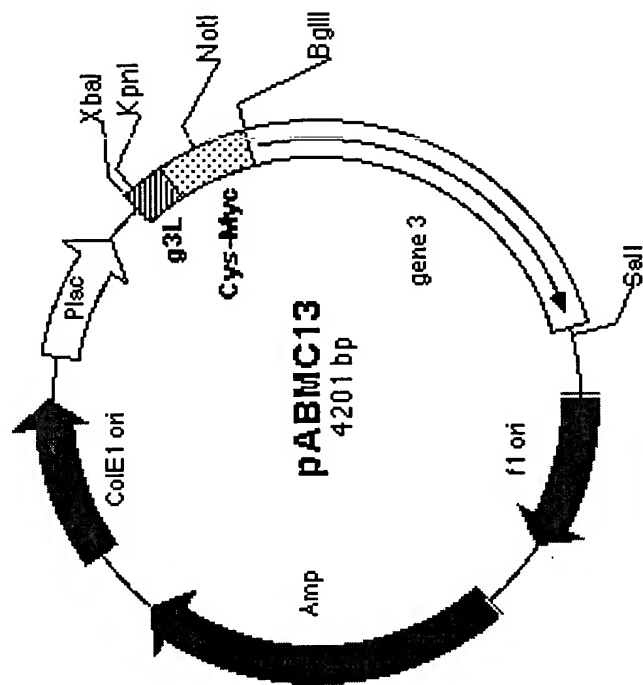


Fig. 12

Helper phage with Cys-Myc-pIII fusion gene

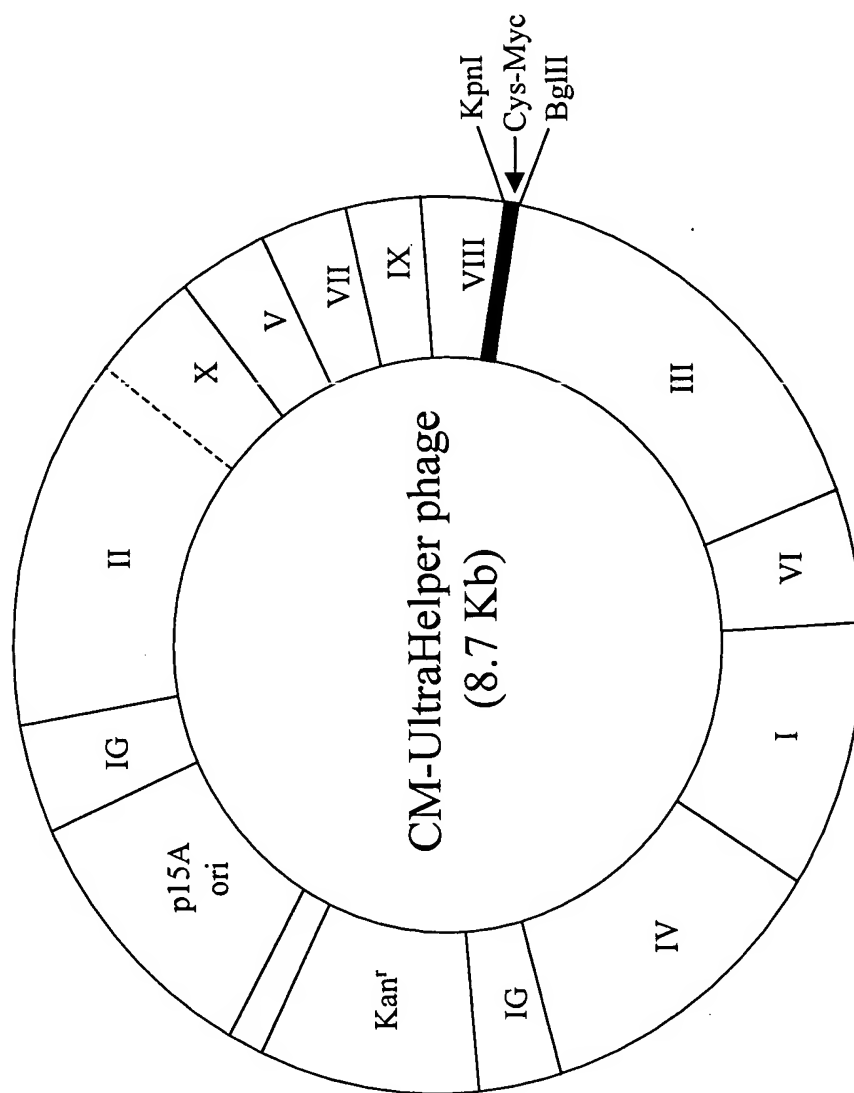


Fig. 13A

TEOTET" 66EEEDOT

Engineered gene III sequence in CM phage

KpnI	Gene III leader	Amber stop	NotI	Myc-tag	BglII
---	<u>TTAGTGGTACCTTTCTATTCTCACTCCGCT</u>	<u>TAGGCTTGC</u>	<u>GGTGGTGGCGGCAGAACAAAACTCATCTCAGAAAGAGGATCTGAGATCT</u>	<u>AGATCTGGA</u>	
- L	V P F Y S H S A *	A C G G A A A E Q K L I S E E D L R S R S G			

Gene III

GGCGGT	ACTGTTGAAAGTTGTTTAGCAAAACCTCATA	CAGAAAATTTCATTACTTAACGTC	TGGAAAGACGACAAAACTTTAGATCGTTACGCT	-----
G G	T V E S C L A K P H T E N S F T N V W K D K T L D R Y A			- - -

Fig. 13B

Detection of Myc-tag on CM-UltraHelper phages by ELISA

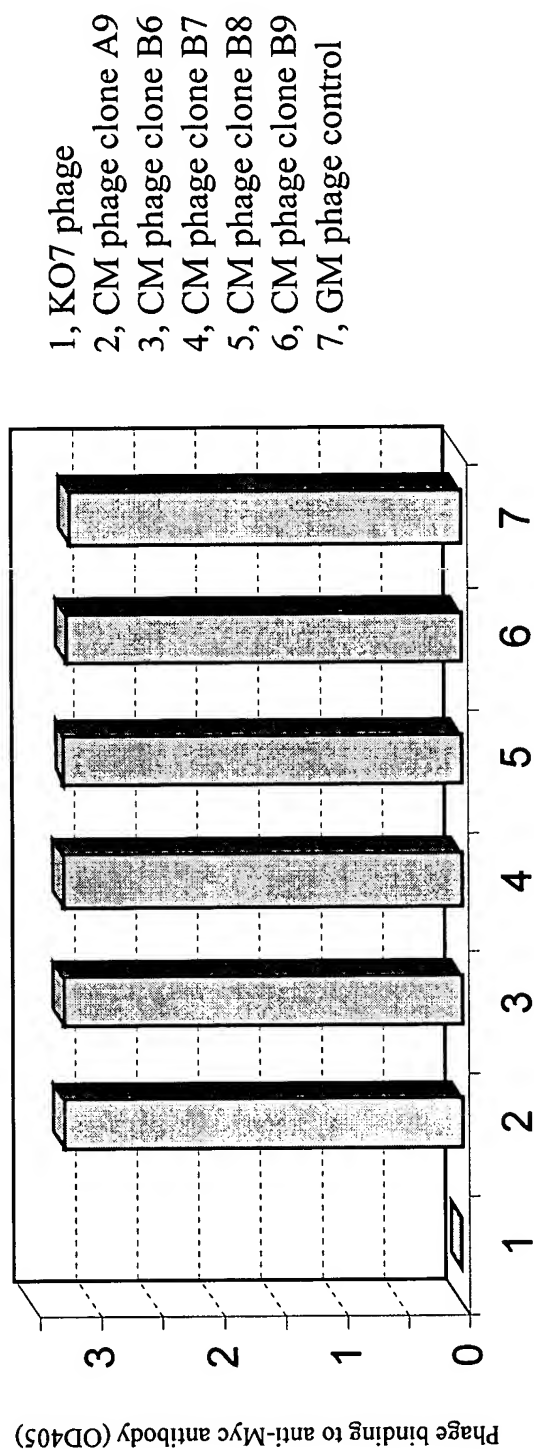


Fig. 14

Phagemid vector for protein-HA-cys expression

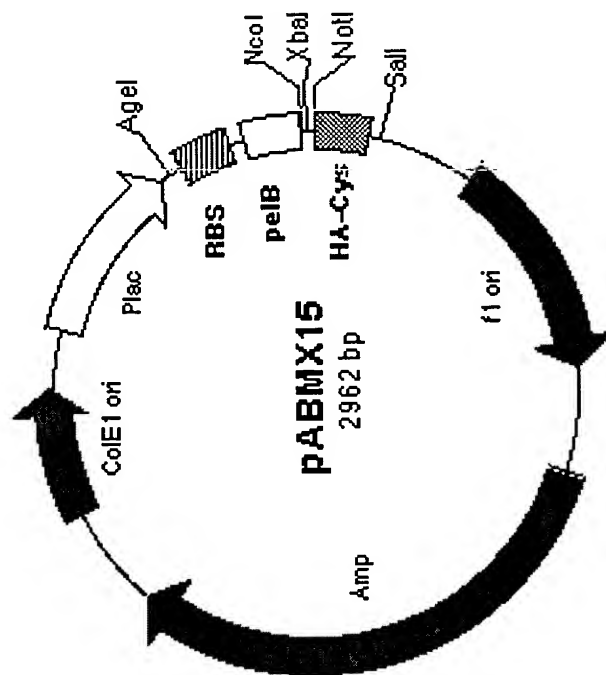


Fig. 15A

[illegible]

Fiio 15B

Functional display of scFv by CM-UltraHelper phage

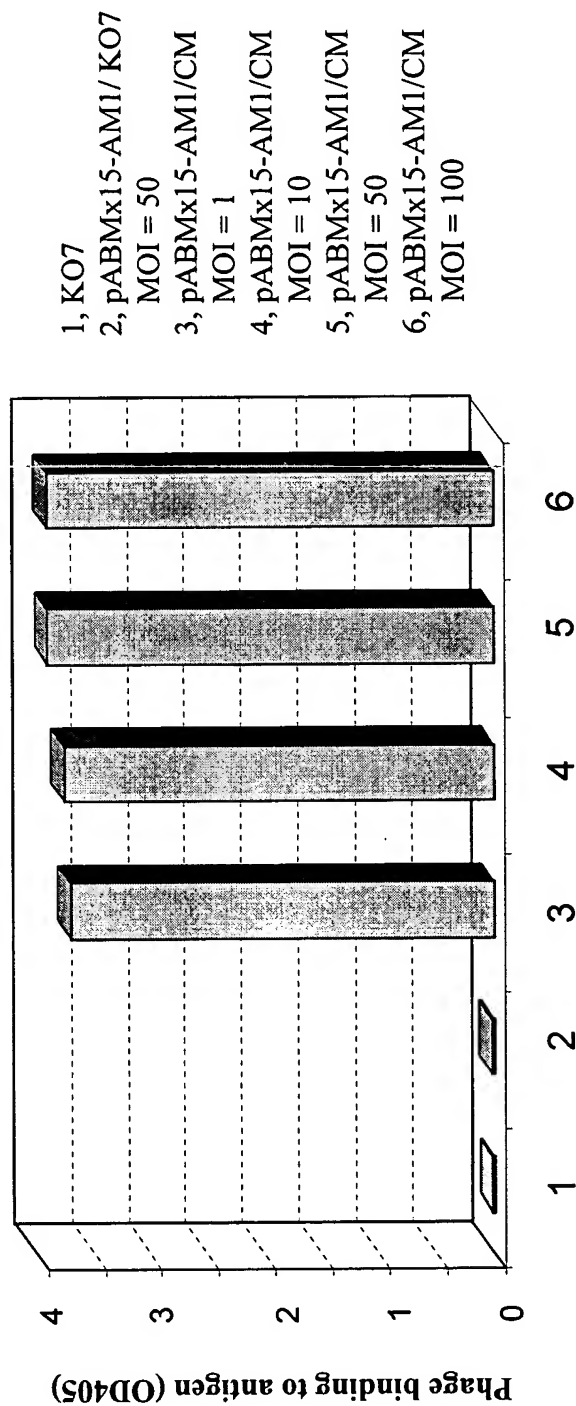
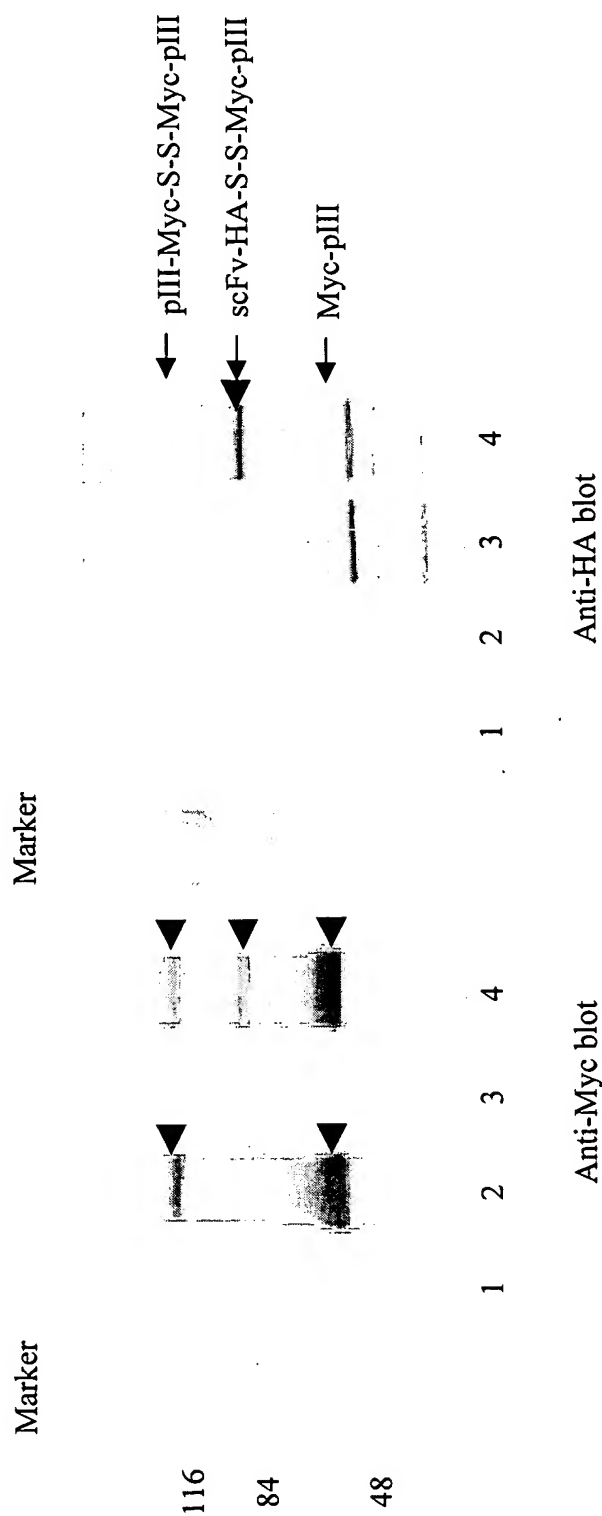


Fig. 16

Detection of scFv displayed by CM-UltraHelper phage



1: KO7 phage; 2: CM phage; 3: pABMx15-AM1/KO7; 4: pABMx15-AM1/CM

Fig. 17

Map of phagemid vector pABMC12

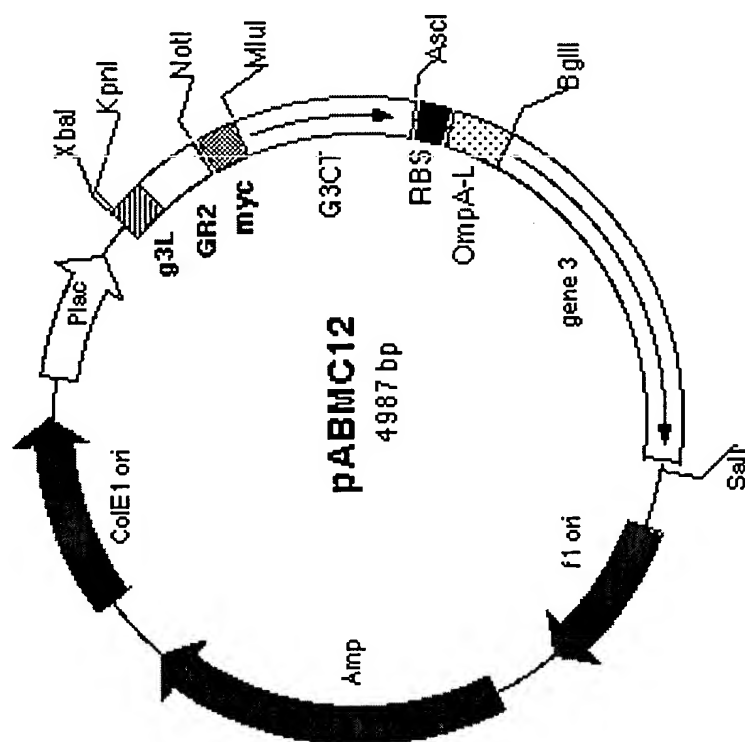


Fig. 18

Helper phage with an additional copy of engineered gene III

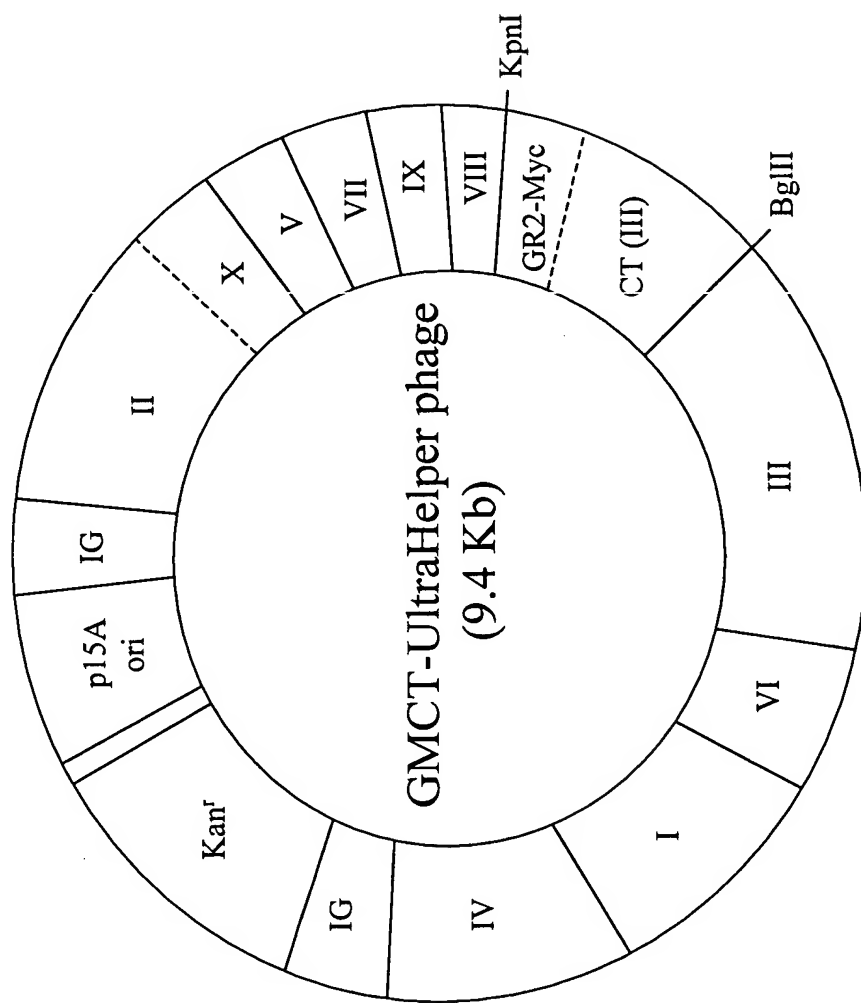


Fig. 19A

Functional display of scFv by GMCT-UltraHelper phage

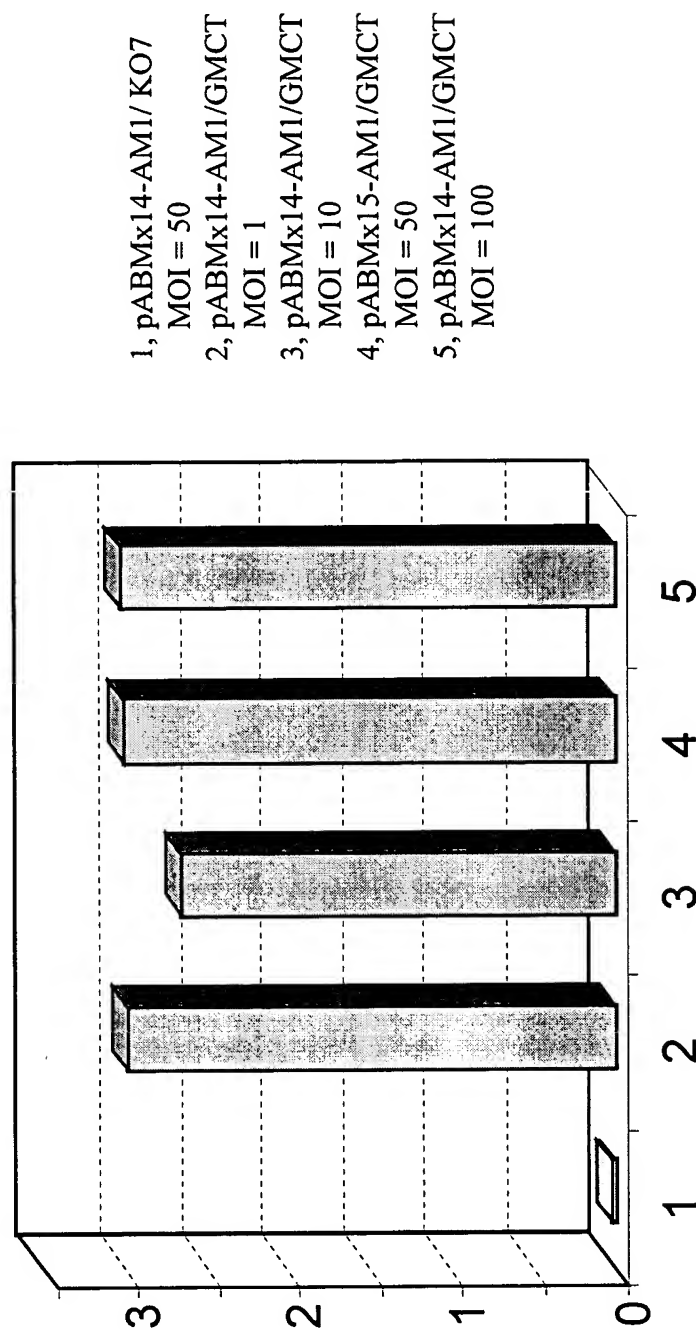


Fig. 20

Detection of scFv displayed by GMCT-UltraHelper phage

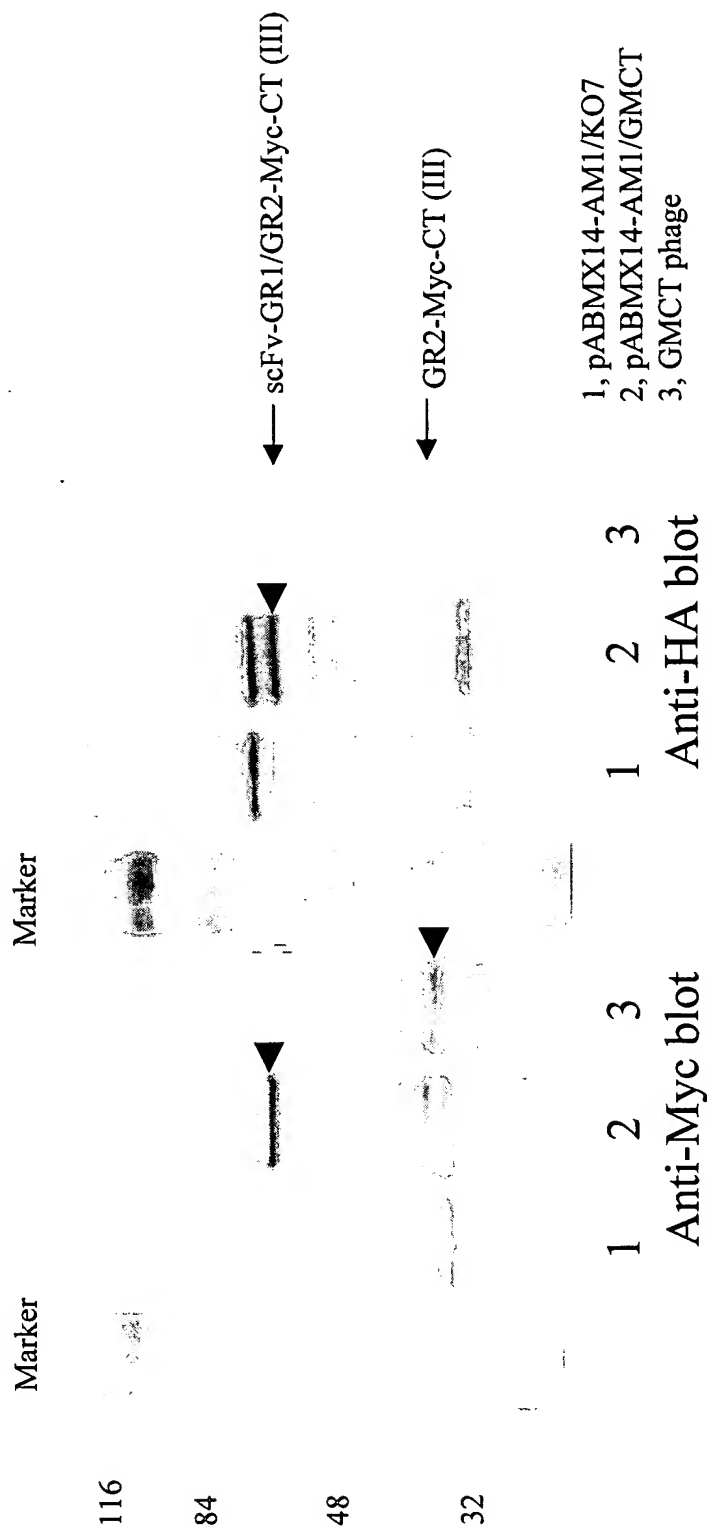


Fig. 21

1039

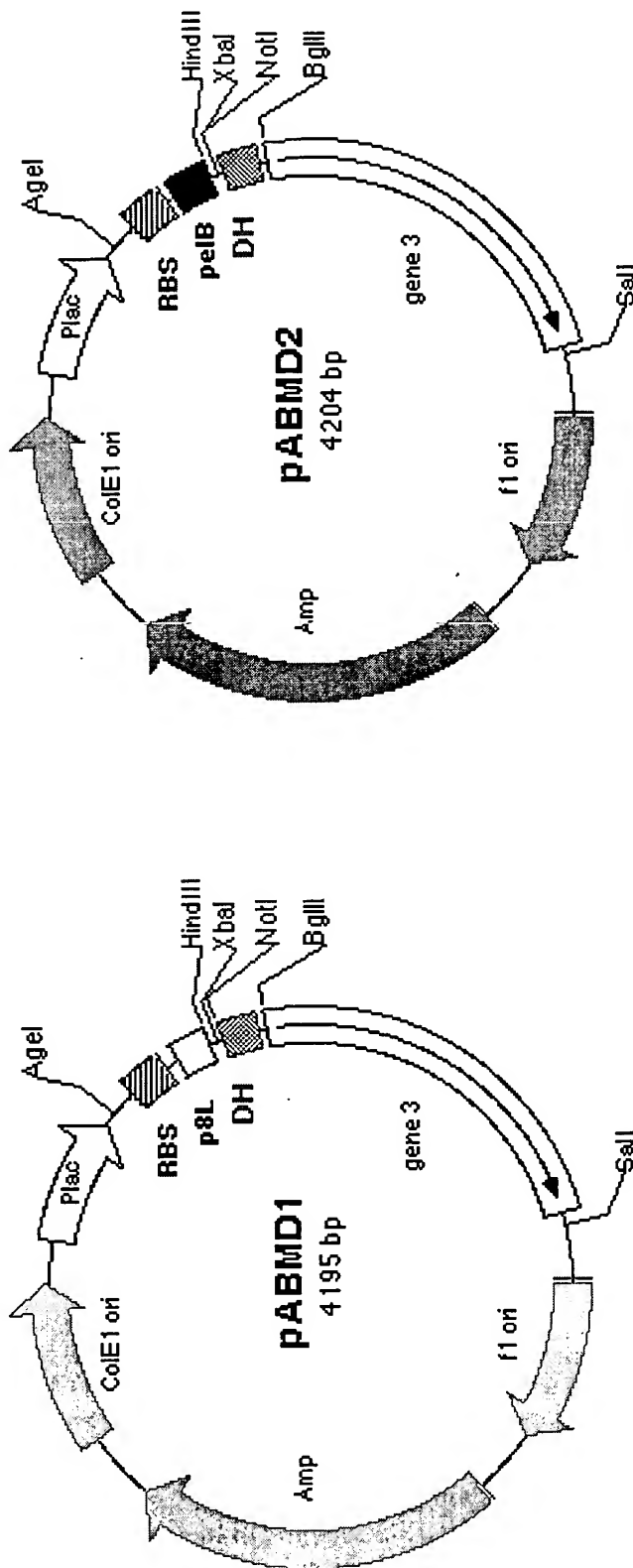


Fig. 22A

PABMD1 vector: sequence from AgeI to SalI

```

lac promoter/lac O1      AgeI      EP      S/D
AATTGTGAGCGGATAACAATT ACCGGT TCCT TTAACCTTAG TAAGGAGG AATTAAAAA
                                HindIII
ATGAAAAAGTCTTTAGTCTCAAAGCCTCCGTAGCCGTTGCTACCTCGTTCGATGCTAAGCTTCGCT TCTAGA
M K K S L V L K A S V A V A T L V P M L S F A S R
                                NotI
GCGGCCGCT TATCCATACGACGTACCGACTACGCA GGAGGT CATCACCATCATCACCAT TAG AGATCT
A A A Y P Y D V P D Y A G G H H H H H * R S
                                His-tag
GGAGGCCGT ACTGTTGAAAAGTTGTTTAGCAAAA ---- GCTAACATACCTGCGTAATAAGGAGTCTTAA GTCGAC
G G G T V E S C L A K ---- A N I L R N K E S *
                                Sall

```

PABMD2 vector: sequence from AgeI to SalI

```

lac promoter/lac O1      AgeI      EP      S/D
AATTGTGAGCGGATAACAATT ACCGGT TCCT TTAACCTTAG TAAGGAGG AATTAAAAA
                                NcoI
ATGAAATACCTATTGCCCTACGGCAGCCGCTGGATTGTTATTACTCGCGGCCAGCCGCCATGGCGGCCCTGCAGGCCCTCTAGA
M K Y L L P T A A A G L L L L A A Q P A M A A L Q A S R
                                PstI
GCGGCCGCT TATCCATACGACGTACCGACTACGCA GGAGGT CATCACCATCATCACCAT TAG AGATCT
A A A Y P Y D V P D Y A G G H H H H H * R S
                                His-tag
GGAGGCCGT ACTGTTGAAAAGTTGTTTAGCAAAA ---- GCTAACATACCTGCGTAATAAGGAGTCTTAA GTCGAC
G G G T V E S C L A K ---- A N I L R N K E S *
                                Sall

```

Fig. 22B

GR1 Sequence Range: 1 to 146

XbaI	10	20	30	40	50
	<u>TCTAGAGGTGGAGGAGGTGAGGAGAAGTCCCGGCTGTGGAGAAGGAGAA</u>				
	S	R	G	G	E
	60	70	80	90	100
	CCGTGAACTGGAAAAGATCATTTGCTGAGAAAAGAGGAGCGTGTCTCTGAAC				
	R	E	L	E	K
	110	120	130	140	150
	TGCGCCATCAACTCCAGTCTGTAGGAGGTGTGTTAATAGGGCGCGCC				
	L	R	H	Q	L
	160	170	180	190	200
	L R H Q L Q S V G G C * *				

GR2 Sequence Range: 1 to 140

XhoI	10	20	30	40	50
	<u>TCTCGAGGAGGTGGTGAACATCCCGCCTGGAGGGCTACAGTCAGAAAA</u>				
	S	R	G	G	E
	60	70	80	90	100
	CCATCGCCTGCGAATGAAGATCACAGAGCTGGATAAAGACTTGAAGAGG				
	H	R	L	R	M
	110	120	130	140	150
	TCACCATGCAGCTGCAGGACGTGCGAGGTGCGCGCGCGC				
	V	T	M	Q	L
	160	170	180	190	200
	V T M Q L Q Q D V G G C A A A				

Fig. 23

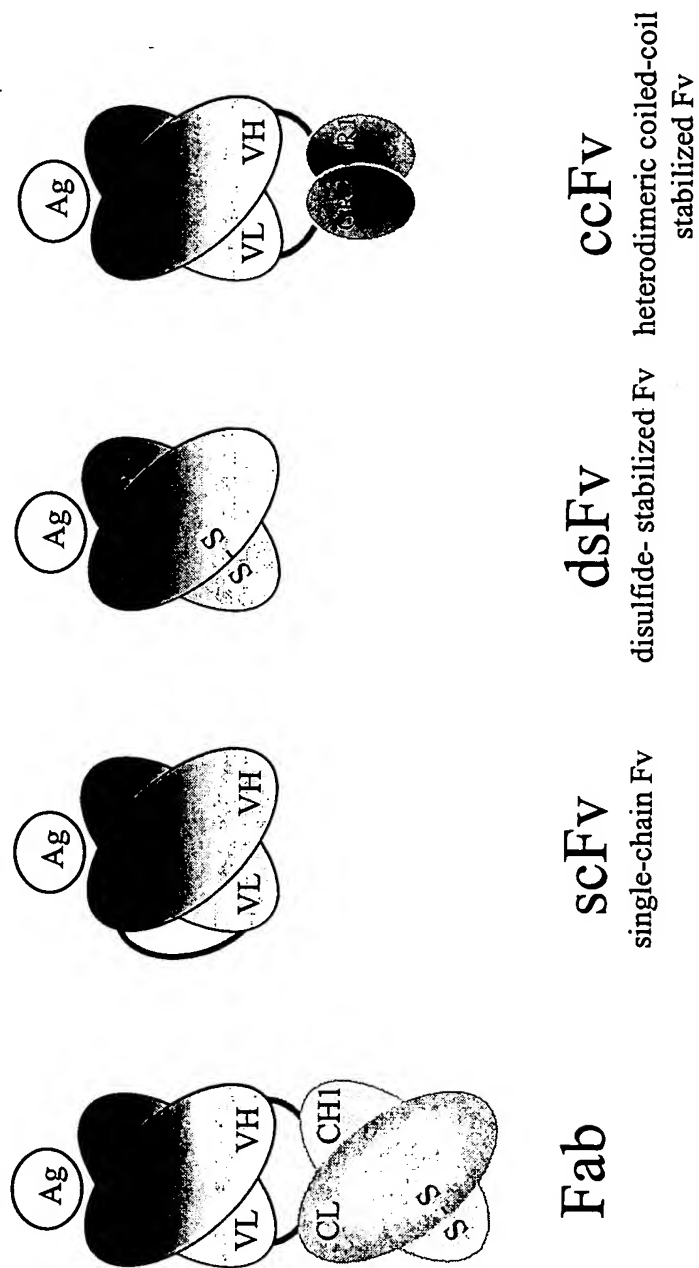


Fig. 24

Expression vector for Adapter-directed bacterial display

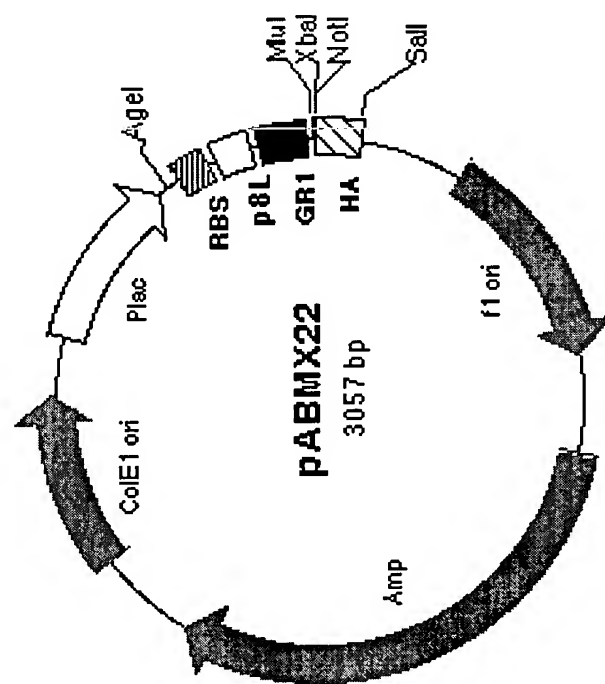


Fig. 25A

Complete vector sequence of pABMX22

[illegible]

Fig. 25B

Helper vector for adapter-directed bacterial display

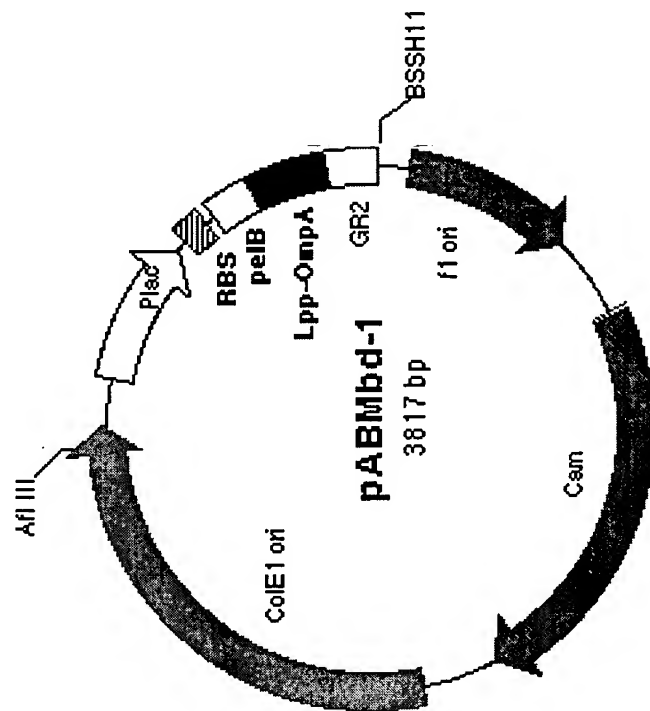


Fig. 26A

Complete vector sequence of pABMXbd-1